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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/19, C07K 14/52, C12N 1/21, C07K 16/24, C12Q 1/68, G01N 33/68, C12N 15/11

A1

(11) International Publication Number: WO 96/22374

(43) International Publication Date: 25 July 1996 (25.07.96)

(21) International Application Number: PCT/US96/00920

(22) International Filing Date: 19 January 1996 (19.01.96)

(30) Priority Data:
08/375,346 19 January 1995 (19.01.95) US

(71) Applicant *(for all designated States except US)*: INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: COLEMAN, Roger [US/US]; 260 Mariposa #2, Mountain View, CA 94041 (US). WILDE, Craig, G. [US/US]; 1239 Mandarin Drive, Sunnyvale, CA 94087 (US). SEILHAMER, Jeffrey, J. [US/US]; 12555 La Cresta Drive, Los Altos Hills, CA 94022 (US).

(74) Agents: LUTHER, Barbara, J. et al.; Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: A NEW CHEMOKINE EXPRESSED IN FETAL SPLEEN, ITS PRODUCTION AND USES

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                157                 166                 175                 184                 193                 202
5' ATG GCC CTG CTA CTG GCC CTC AGC CTG CTG GTT CTC TGG ACT TCC CCA GCC CCA
M  A   L   L   L   A   L   S   L   L   V   L   W   T   S   P   A   P

                211                 220                 229                 238                 247                 256
ACT CTG AGT GGC ACC AAT GAT GCT GAA GAC TGC TGC CTG TCT GTG ACC CAG AAA
T   L   S   G   T   N   D   A   E   D   C   C   L   S   V   T   Q   K

                265                 274                 283                 292                 301                 310
CCC ATC CCT GGG TAC ATC GTG AGG AAC TTC CAC TAC CTT CTC ATC AAG GAT GGC
P   I   P   G   Y   I   V   R   N   F   H   Y   L   L   I   K   D   G

                319                 328                 337                 346                 355                 364
TGC AGG GTG CCT GCT GTA GTG TTC ACC ACA CTG AGG GGC CGC CAG CTC TGT GCA
C   R   V   P   A   V   V   F   T   T   L   R   G   R   Q   L   C   A

                373                 382                 391                 400                 409                 418
CCC CCA GAC CAG CCC TGG GTA GAA CGC ATC ATC CAG AGA CTG CAG AGG ACC TCA
P   P   D   Q   P   W   V   E   R   I   I   Q   R   L   Q   R   T   S

                427                 436                 445
GCC AAG ATG AAG CGC CGC AGC AGT TAA 3'
A   K   M   K   R   R   S   S   *
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(57) Abstract

The present invention provides nucleotide and amino acid sequences that identify and encode a novel chemokine (FSEC) initially found in human fetal spleen cells. The present invention also provides for antisense molecules to the nucleotide sequences which encode FSEC, expression vectors for the production of purified FSEC, antibodies capable of binding specifically to FSEC, hybridization probes or oligonucleotides for the detection of FSEC-encoding nucleotide sequences, genetically engineered host cells for the expression of FSEC, diagnostic tests for chemokine activation based on FSEC-encoding nucleic acid molecules and antibodies capable of binding specifically to FSEC.

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A NEW CHEMOKINE EXPRESSED IN FETAL SPLEEN, ITS PRODUCTION AND USES

BACKGROUND ART

The chemokines are a family of cytokines that are produced when the immune system responds to non-self antigens, such as invading microorganisms or antigens of an incompatible tissue type and are associated with leukocyte trafficking in abnormal, inflammatory or diseased conditions. Chemokines mediate the expression of particular adhesion molecules on endothelial cells and they generate gradients of chemoattractant factors which activate specific cell types. In addition, the chemokines stimulate the proliferation of specific cell types and regulate the activation of cells which bear specific receptors. These activities demonstrate a high degree of target cell specificity.

The chemokines are small polypeptides, generally about 70-100 amino acids in length, 8-11 kD in molecular weight and active over a 1-100 ng/ml concentration range. Initially, they were isolated and purified from inflamed tissues and characterized relative to their bioactivity. More recently, chemokines have been discovered through molecular cloning techniques and characterized by structural as well as functional analysis.

The chemokines are related through a four-cysteine motif which is based primarily on the spacing of the first two cysteine residues in the mature molecule. Currently the chemokines are assigned to one of two families, the C-C chemokines (α) and the C-X-C chemokines (ß). Although exceptions exist, the C-X-C chemokines generally activate neutrophils and fibroblasts while the C-C chemokines act on a more diverse group of target cells which include monocytes/macrophages, basophils, eosinophils, T lymphocytes and others. The known chemokines of both families are synthesized by many diverse cell types as reviewed in Thomson A. (1994) The Cytokine Handbook, 2d Ed. Academic Press, New York City. The two groups of chemokines will be described in turn.

C-C chemokines appear to have less N-terminal processing than the C-X-C chemokines. Known human and/or murine C-C chemokines include MIP-1α and ß; I-309; RANTES and MCP-1. The macrophage inflammatory proteins alpha and beta (MIP-1α and ß) were first purified from

stimulated mouse macrophage cell line and elicited an inflammatory response when injected into normal tissues. At least three distinct and non-allelic genes encode human MIP-1α, and seven distinct genes encode MIP-1ß.

MIP-1α and MIP-1ß consist of 68-69 amino acid which are about 70% identical in their acidic, mature secreted forms. They are both expressed in stimulated T cells, B cells and monocytes in response to mitogens, anti-CD3 and endotoxin, and both polypeptides bind heparin. While both molecules stimulate monocytes, MIP-1α chemoattracts the CD-8 subset of T lymphocytes and eosinophils, while MIP-1ß chemoattracts the CD-4 subset of T lymphocytes. In mouse, these proteins are known to stimulate myelopoiesis.

I-309 was cloned from a human γ-δ T cell line and shows 42% amino acid identity to T cell activation gene 3 (TCA3) cloned from mouse. There is considerable nucleotide homology between the 5' flanking regions of these two proteins, and they share an extra pair of cysteine residues not found in other chemokines. Such similarities suggest I-309 and TCA3 are species homologs which have diverged over time in both sequence and function.

RANTES is another C-C chemokine which is expressed in T cells (but not B cells), in platelets, in some tumor cell lines, and in stimulated rheumatoid synovial fibroblasts. In the latter, it is regulated by interleukins-1 and -4, transforming nerve factor and interferon-γ. The cDNA cloned from T cells encodes a basic 8 kD protein which lacks N-linked glycosylation and is able to affect lymphocytes, monocytes, basophils and eosinophils. The expression of RANTES mRNA is substantially reduced following T cell stimulation.

Monocyte chemotactic protein (MCP-1) is a 76 amino acid protein which appears to be expressed in almost all cells and tissues upon stimulation by a variety of agents. The targets of MCP-1, however, are limited to monocytes and basophils in which it induces a MCP-1 receptor:G protein-linked calcium flux (Charo I, personal communication). Two other related proteins (MCP-2 and MCP-3) were purified from a human osteosarcoma cell line. MCP-2 and MCP-3 have 62% and 73% amino acid identity, respectively, with MCP-1 and share its chemoattractant specificity for monocytes.

The chemokine molecules have been reviewed in Schall TJ

(1994) Chemotactic Cytokines: Targets for Therapeutic Development. International Business Communications, Southborough, MA, pp 180-270; and in Paul WE (1993) Fundamental Immunology, Raven Press, New York City (NYC), pp 822-826.

In humans, the spleen clears the blood of microorganisms and particulate antigens and/or generates antigens to foreign substances; sequesters and removes excess, old and/or abnormal blood cells; regulates portal blood flow and engages in hematopoiesis during development or when the bone marrow alone cannot produce sufficient blood cells.

In the spleen as well as other tissues, leukocytes including monocytes, macrophages, basophils, and eosinophils play important roles in the pathological mechanisms initiated by T and/or B lymphocytes. Macrophages, in particular, produce powerful oxidants and proteases which contribute to tissue destruction and secrete a range of cytokines which recruit and activate

Current techniques for diagnosis of abnormalities in the inflamed or diseased tissues mainly rely on observation of clinical symptoms or serological analyses of body tissues or fluids for hormones, polypeptides or various metabolites. Patients often manifest no clinical symptoms at early stages of disease or tumor development. Furthermore, serological analyses do not always differentiate between invasive diseases and genetic syndromes which have overlapping or very similar ranges. Thus, development of new diagnostic techniques comprising the use of chemokines would provide for early and accurate diagnoses of disease states and associated conditions, would provide a better understanding of molecular pathogenesis, and would provide the basis for development of effective therapies.

DISCLOSURE OF THE INVENTION

The subject invention provides a nucleotide sequence which encodes a novel chemokine initially found in human fetal spleen tissue. The new gene, which is known as fetal spleen expressed chemokine, or fsec (Incyte Clone 29592), encodes a polypeptide designated FSEC, of the C-C chemokine family. The present invention relates to diagnostic tests for physiologic or pathologic compromise of the spleen or other tissues where

FSEC is associated with a disease state which include steps for testing a sample or an extract thereof with fsec DNA, fragments or oligomers thereof. Aspects of the invention include fsec antisense molecules; cloning or expression vectors containing nucleic acid encoding fsec; host cells or organisms transformed with expression vectors containing nucleic acid encoding fsec; purified FSEC and methods for the production and recovery of FSEC from host cells. The present invention also relates to treatment of disease states and conditions associated with FSEC, such as inflammation.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 displays the nucleotide sequence for fetal spleen expressed chemokine (fsec) and the predicted amino acid sequence of fetal spleen expressed chemokine, FSEC (SEQ ID NO: 1 and SEC ID NO:2, respectively).

Figure 2 shows the amino acid alignment of FSEC with other human chemokines of the C-C family. Alignments shown were produced using the multisequence alignment program of DNASTAR software (DNASTAR Inc, Madison WI).

Figure 3 displays an analysis of FSEC hydrophobicity based on the predicted amino acid sequence and composition.

Figure 4 shows a relatedness tree of human C-C chemokines. The phylogenetic tree was generated by phylogenetic tree program of DNASTAR software (DNASTAR Inc, Madison WI) using the Clustal method with the PAM250 residue weight table.

MODES FOR CARRYING OUT THE INVENTION

Definitions

As used herein, the term "fetal spleen expressed chemokine" or "FSEC", refers to the polypeptide disclosed in SEQ ID NO:2, or an active fragment thereof, which is encoded by an mRNA transcribed from fsec cDNA of SEQ ID NO:1. FSEC may be naturally occurring or chemically synthesized. As used herein, the lower case "fsec" refers to a nucleic acid sequence while an upper case "FSEC" refers to a protein, peptide or amino acid sequence.

As used herein the term "active" refers to those forms of FSEC which retain the biologic and/or immunologic activities of naturally occurring FSEC.

As used herein the term "naturally occurring FSEC" refers to FSEC produced by human cells that have not been genetically engineered and specifically contemplates various FSEC forms arising from post-translational modifications of the polypeptide including but not limited to acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

As used herein the term "derivative" refers to polypeptides derived from naturally occurring FSEC by chemical modifications such as ubiquitination, labeling (e.g., with radionuclides, various enzymes, etc.), pegylation (derivatization with polyethylene glycol) or by insertion or substitution by chemical synthesis of amino acid such as ornithine, which do not normally occur in human proteins.

As used herein the term "variant" or "recombinant variant" or "mutant" refers to any polypeptide differing from naturally occurring FSEC by amino acid insertions, deletions, and substitutions, created using recombinant DNA techniques. Guidance in determining which amino acid residues may be replaced, added or deleted without abolishing activities of interest, such as cell adhesion and chemotaxis, may be found by comparing the sequence of the particular FSEC with that of homologous cytokines and minimizing the number of amino acid sequence changes made in regions of high homology or regions of known activity.

Preferably, amino acid "substitutions" are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine, i.e., conservative amino acid replacements. "Insertions" or "deletions" are typically in the range of about 1 to 5 amino acids. The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acid in FSEC using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

Where desired, nucleic acid encoding FSEC or and FSEC variant can be genetically engineered to contain a "signal or leader sequence" that can direct the polypeptide through the membrane of a cell. As will be understood by one of skill in the art, such a sequence may be naturally occurring on the polypeptides of the present invention or provided from heterologous protein sources by recombinant DNA techniques.

As used herein, an FSEC "fragment," "portion," or "segment" refers is a stretch of amino acid residues which has sufficient length to display biologic and/or immunogenic activity and in preferred embodiments will contain at least about 5 amino acids, at least about 7 amino acids, at least about 8 to 13 amino acids, and, in additional embodiments, about 17 or more amino acids.

As used herein, an "oligonucleotide" or polynucleotide "fragment", "portion," or "segment" refers to any stretch of nucleic acids encoding FSEC which is of sufficient length to use as a primer in polymerase chain reaction (PCR) or various hybridization procedures known to those of skill in the art, for the purpose of identifying or amplifying identical or related nucleic acids.

The present invention includes purified FSEC polypeptides from natural or recombinant sources, vectors and host cells transformed with recombinant nucleic acid molecules encoding FSEC. Various methods for the isolation of the FSEC polypeptides may be accomplished by procedures well known in the art. For example, such polypeptides may be purified by immunoaffinity chromatography by employing the antibodies provided by the present invention. Various other methods of protein purification well known in the art include those described in Deutscher M (1990) Methods in Enzymology, Vol 182, Academic Press, San Diego; and Scopes R (1982) Protein Purification: Principles and Practice. Springer-Verlag, NYC, both incorporated herein by reference.

As used herein the term "recombinant" refers to a polynucleotide which encodes FSEC and is prepared using recombinant DNA techniques. The polynucleotide which encodes FSEC may also include allelic or recombinant variants and mutants thereof.

As used herein the term "probe" or "nucleic acid probe" or "oligonucleotide probe" refers to a portion, fragment, or segment of fsec that is capable of being hybridized to a desired target nucleotide sequence. A probe can be used to detect, amplify or quantify cDNAs or endogenous nucleic acid encoding FSEC by employing conventional techniques in molecular biology. A probe may be of variable length, preferably from about 10 nucleotides up to several hundred nucleotides. As will be understood by those of skill in the art, hybridization conditions and probe design will vary depending upon the intended use. For example, a probe intended for use

in PCR will be from about 15 to 30 nucleotides in length and may be part of a pool of degenerate probes, i.e., oligonucleotides which tolerate nucleotide mismatch but accommodate binding to an unknown sequence; whereas a probe for use in Southern or northern hybridizations may be a single, specific nucleotide sequence that is several hundred nucleotides in length. Accordingly, a preferred probe for the specific detection of fsec will comprise a polynucleotide or oligonucleotide fragment from a non-conserved nucleotide region of SEQ ID NO:1. As used herein the term "non-conserved nucleotide region" refers to a nucleotide region that is unique to SEQ ID NO:1 and does not comprise a region that is conserved in the family of C-C chemokines. Probes may be single-stranded or double-stranded and may have specificity in solution, cell, tissue or membrane-based hybridizations including in situ and ELISA-like technologies. The present invention encompasses oligonucleotides, fragments or portions of the polynucleotides disclosed herein, or their complementary strands used as probes. In the present invention, "oligonucleotides" or "oligonucleotide probes" are based on the nucleotide sequences disclosed herein which encode FSEC. Oligonucleotides comprise portions of the nucleotide sequence disclosed herein and contain at least about 15 nucleotides, and usually at least about 20 nucleotides and may include up to 60 nucleotides. Nucleic acid probes may comprise portions of the sequence having fewer nucleotides than about 6 kb and usually fewer than about 1 kb. The oligonucletides and nucleic acid probes of the present invention may be used to determine whether nucleic acid encoding FSEC is present in a cell or tissue or to isolate identical or similar nucleic acid sequences from chromosomal DNA as described by Walsh PS et al (1992 PCR Methods Appl 1:241-250).

Nucleic acid probes of the present invention may be derived from naturally occurring or recombinant single- or double-stranded nucleic acids or be chemically synthesized. They may be labeled by nick translation, Klenow fill-in reaction, PCR or other methods well known in the art. Probes of the present invention, their preparation and/or labeling are elaborated in Sambrook J et al (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY; or Ausubel FM et al (1989) Current Protocols in Molecular Biology, John Wiley & Sons, NYC, both incorporated herein by reference.

Alternatively, recombinant variants encoding the

polypeptides of the present invention or related polypeptides may be synthesized or identified through hybridization techniques known to those of skill in the art by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic system. Mutations may also be introduced to modify the properties of the polypeptide, to change ligand-binding affinities, interchain affinities, or polypeptide degradation or turnover rate.

Detailed Description of the Invention

The present invention provides a nucleotide sequence uniquely identifying FSEC, a novel chemokine of the C-C family, which was initially found expressed in fetal spleen tissue and additionally found in inflamed adenoid tissue, thymus tissue, elbow synovium, breast tissue, kidney tissue from an infant who died of anoxia, and spinal cord tissue from an individual who died of respiratory distress. The use of FSEC and the nucleic acid sequences which encode it, is based on its characterization as a C-C chemokine and the function of known C-C chemokines in activating monocytes, macrophages, basophils, eosinophils, T lymphocytes and/or other cells which respond by producing abundant proteases and other molecules which can lead to tissue damage or destruction. The present invention is also based in part on the expression of FSEC in inflamed tissues, such as inflamed adenoid tissue, and likely expression in inflamed cells in other tissues having immune involvement, such as in synovium, spleen and thymus, and expression in cells of stressed tissues.

Accordingly, nucleic acid sequences which encode FSEC and anti-FSEC antibodies provide the basis for diagnosing diseases or conditions related to activation of monocytes, macrophages, basophils, eosinophils, T lymphocytes and/or other cells which respond by producing abundant proteases and other molecules which can lead to tissue damage or destruction. A diagnostic test specific for the expression of FSEC can accelerate diagnosis of FSEC associated disease states thereby providing an opportunity for early treatment of such disease states before extensive tissue damage or destruction occurs.

The present invention also relates to the use of nucleic acid

sequences encoding FSEC and anti-FSEC antibodies to treat or ameliorate the symptoms of disease states or conditions associated with excess expression of FSEC or associated with FSEC related activation of monocytes, macrophages, basophils, eosinophils, T lymphocytes and/or other cells which respond by producing abundant proteases. Such diseases or conditions include but are not limited to, diseases or conditions related to inflammation, such as viral or bacterial infection, including mononucleosis and malaria; diseases affecting immunoregulation, such as the auto-immune diseases rheumatoid arthritis or systemic lupus erythematosis; AIDS associated inflammation; inflammation associated with disease of erythrocytes or blood flow such as sickle cell anemia and β thalassemia; and other physiologic and pathologic problems related to excess production of FSEC induced proteases, including mechanical injury associated with trauma. For example, an fsec anti-sense molecule can be administered to an individual subject to FSEC associated inflammation to inhibit the translation of endogenous fsec, thereby ameliorating the symptoms of inflammation.

The present invention also relates to the use of FSEC or nucleic acid sequences encoding FSEC to treat or ameliorate the symptoms of disease states or conditions where the immune system is comprised, such as in AIDS where administration of FSEC or nucleic acid encoding FSEC would replace or augment naturally occuring FSEC and function to activate and attract other molecules of the immune system.

The nucleotide sequences of the present invention encoding FSEC, or their complements, have numerous applications in techniques known to those skilled in the art of molecular biology. These techniques include the use of FSEC nucleotide sequences as hybridization probes to detect nucleotide sequences encoding FSEC in biological samples; use as oligomers in PCR to identify and/or amplify nucleotide sequences encoding FSEC; use for chromosome and gene mapping, use in the recombinant production of FSEC, and use in the generation of anti-sense DNA or RNA which may inhibit translation of FSEC.

Accordingly, the present invention provides a diagnostic test for the detection of nucleotide sequences encoding FSEC in a biological sample, comprising the steps of combining the biological sample with a first nucleotide which comprises the nucleotide sequence of SEQ ID NO:1 or a

fragment thereof, wherein said fragment is derived from a non-conserved region of said nucleotide, under conditions suitable for the formation of a nucleic acid hybridization complex; detecting said hybridization complex wherein the presence of said complex correlates with the presence of a second nucleotide encoding FSEC in said biological sample; and comparing the amount of the second nucleotide in said sample with a standard thereby determining whether the amount of said second nucleotide varies from the standard, wherein the presence of an abnormal level of said second nucleotide correlates positively with a condition associated with aberrant expression of FSEC. The first nucleotide can be labeled with a reporter molecule allowing the hybridization complex to be detected by measuring the reporter molecule.

Additionally, the present invention also provides a diagnostic test for the detection of nucleotide sequences encoding FSEC in a biological sample, comprising the steps of combining the biological sample with polymerase chain reaction primers under conditions suitable for nucleic acid amplification, wherein said primers comprise fragments from non-conserved regions of the nucleotide sequence of SEQ ID NO:1; detecting amplified nucleotide sequences; and comparing the amount of amplified nucleotide sequences in said biological sample with a standard thereby determining whether the amount of said nucleotide sequence varies from said standard, wherein the presence of an abnormal level of said nucleotide sequence correlates positively with a condition associated with aberrant expression of FSEC.

The present invention also provides methods of screening for drugs or other agents which can specifically bind FSEC thereby identifying potential therapeutics which may treat or ameliorate the symptoms of diseases or conditions associated with aberrant expression of FSEC. Accordingly, the present invention provides a method for screening a plurality of compounds for specific binding affinity with FSEC or any portion thereof, comprising the steps of providing a plurality of compounds; combining FSEC with each of a plurality of compounds for a time sufficient to allow binding under suitable conditions; and detecting binding of FSEC to each of the plurality of compounds, thereby identifying the compounds which specifically bind FSEC.

Uses of nucleotides encoding FSEC disclosed herein are

exemplary of known techniques and are not intended to limit their use in any technique known to a person of ordinary skill in the art. Furthermore, the nucleotide sequences disclosed herein may be used in molecular biology techniques that have not yet been developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, e.g., for example, the triplet genetic code, specific base pair interactions, etc.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of FSEC-encoding nucleotide sequences, some bearing minimal homology to the nucleotide sequence of any known and naturally occurring gene may be produced. The invention has specifically contemplated each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of naturally occurring FSEC and may be produced as long as the nucleotide sequence encodes FSEC disclosed in SEQ ID NO:2, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode FSEC and/or FSEC variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring FSEC gene under stringent conditions, it may be advantageous to produce nucleotide sequences encoding FSEC or FSEC derivatives possessing a substantially different codon usage. Codons can be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic expression host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding FSEC and/or FSEC derivatives without altering the encoded aa sequence include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

Nucleotide sequences encoding FSEC may be joined to a variety of other nucleotide sequences by means of well established recombinant DNA techniques (cf Sambrook J et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY). Useful nucleotide sequences for joining to fsec include an assortment of cloning vectors, e.g., plasmids, cosmids, lambda phage derivatives, phagemids, and the like, that are well

known in the art. Vectors of interest include expression vectors, replication vectors, probe generation vectors, sequencing vectors, and the like. In general, vectors of interest may contain an origin of replication functional in at least one organism, convenient restriction endonuclease sensitive sites, and selectable markers for the host cell.

Another aspect of the subject invention is to provide for fsec-specific nucleic acid hybridization probes capable of hybridizing with naturally occurring nucleotide sequences encoding FSEC. Such probes for the detection of fsec encoding sequences should preferably contain a nucleotide fragment from a non-conserved region of SEQ ID NO:1. Such probes may also be used for the detection of related chemokine encoding sequences and should preferably contain at least 50% of the nucleotides from a C-C encoding sequence. The hybridization probes of the subject invention may be derived from the nucleotide sequences of the SEQ ID NO:1 or from genomic sequences including promoters, enhancer elements and introns of naturally occurring fsec. The hybridization probes may be labeled by a variety of reporter groups, including radionuclides such as 32P or 35S, or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like through techniques known to those of skill in the art.

PCR as described in United States Patents 4,683,195; 4,800,195; and 4,965,188 provides additional uses for oligonucleotides based upon the nucleotide sequences which encode FSEC. Such probes used in PCR may be of recombinant origin, may be chemically synthesized, or a mixture of both and will comprise either a nucleotide sequence from a non-conserved region of SEQ ID NO:1 for diagnostic use in detecting fsec or a degenerate pool of possible sequences for identification of closely related genomic sequences.

Other means of producing specific hybridization probes for fsec include the cloning of nucleic acid sequences encoding FSEC and FSEC derivatives into vectors for the production of mRNA probes. Such vectors are known in the art and are commercially available and may be used to synthesize RNA probes <u>in vitro</u> by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA polymerase and the appropriate radioactively labeled nucleotides.

Nucleic acid encoding FSEC, portions thereof, or FSEC derivatives may be produced entirely by synthetic chemistry, after which the

gene can be inserted into any of the many available DNA vectors using reagents, vectors and cells that are known in the art at the time of the filing of this application. Moreover, synthetic chemistry may be used to introduce mutations into the fsec sequence or any portion thereof. The nucleotide sequence of nucleic acid encoding FSEC can be confirmed through DNA sequencing techniques.

Methods for DNA sequencing are well known in the art. Conventional enzymatic methods employed DNA polymerase Klenow fragment, SEQUENASE® (US Biochemical Corp, Cleveland, OH) or Taq polymerase to extend DNA chains from an oligonucleotide primer annealed to the DNA template of interest. Methods have been developed for the use of both single- and double-stranded templates. The chain termination reaction products were electrophoresed on urea-acrylamide gels and detected either by autoradiography (for radionuclide-labeled precursors) or by fluorescence (for fluorescent-labeled precursors). Recent improvements in mechanized reaction preparation, sequencing and analysis using the fluorescent detection method have permitted expansion in the number of sequences that can be determined per day (using machines such as the Catalyst 800 and the Applied Biosystems 373 DNA sequencer).

The nucleotide sequence of FSEC provides the basis for assays to detect inflammation or diseases associated with abnormal levels of expression of FSEC. The nucleotide sequence can be labeled by methods known in the art and added to a fluid or tissue sample from a patient under hybridizing conditions. After an incubation period, the sample is washed with a compatible fluid which optionally contains a dye if the nucleotide has been labeled with an enzyme or other label or reporter molecule requiring a developer. After the compatible fluid is rinsed off, the dye is quantitated and compared with a standard. If the amount of dye is significantly elevated, the nucleotide sequence has hybridized with the sample, and the assay indicates the presence of inflammation and/or disease.

The nucleotide sequence encoding FSEC can be used to construct hybridization probes for mapping the gene. The nucleotide sequence provided herein may be mapped to a chromosome and specific regions of a chromosome using well known genetic and/or chromosomal mapping techniques. These techniques include <u>in situ</u> hybridization, linkage analysis against known chromosomal markers, hybridization screening with

libraries or flow-sorted chromosomal preparations specific to known chromosomes, and the like. The technique of fluorescent in situ hybridization of chromosome spreads has been described, among other places, in Verma et al (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, NYC.

Fluorescent in situ hybridization of chromosomal preparations and other physical chromosome mapping techniques may be correlated with additional genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of fsec on a physical chromosomal map and a specific disease (or predisposition to a specific disease) can help delimit the region of DNA associated with that genetic disease. The nucleotide sequence of the subject invention may be used to detect differences in gene sequence between normal and individuals subject to a disease or condition.

Nucleotide sequences encoding FSEC may be used to produce purified FSEC using well known methods of recombinant DNA technology. Among the many publications that teach methods for the expression of genes after they have been isolated is Goeddel (1990) Gene Expression Technology, Methods and Enzymology, Vol 185, Academic Press, San Diego. FSEC may be expressed in a variety of host cells, including cells of prokaryotic or eukaryotic origin. Host cells may be from the same species in which fsec nucleotide sequences are endogenous or from a different species. Advantages of producing FSEC by recombinant DNA technology include obtaining adequate amounts of the protein for purification and the availability of simplified purification procedures. FSEC may be expressed as a chimeric protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle WA). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase(Invitrogen, San Diego CA) between the purification domain and the fsec sequence may be useful to facilitate expression of FSEC.

Translation of the fsec cDNA may be accomplished by subcloning the cDNA into an appropriate expression vector and transfecting

this vector into an appropriate expression host. As described in Example VII, a preferred expression vector for the expression and purification of FSEC is one which provides for expression of a fusion protein comprising a FSEC and contains nucleic acid encoding 6 histidine residues followed by thioredoxin and an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal Ion affinity chromotography as described in Porath et al. (1992) Protein Expression and Purification 3:263-281) while the enterokinase cleavage site provides a means for purifying the chemokine from the fusion protein.

The expression vector used for the generation of the cDNA libraries, which contains a promoter for ß-galactosidase upstream of the cloning site, followed by a nucleotide sequence containing the amino-terminal Met and the subsequent 7 residues of ß-galactosidase, followed by a bacteriophage promoter useful for artificial priming and transcription and a number of unique restriction sites, including Eco RI, can also be used for the expression of FSEC.

Induction of the isolated bacterial strain with IPTG using standard methods will produce a fusion protein corresponding to the first seven residues of ß-galactosidase, about 15 residues of "linker", and FSEC encoded within the cDNA. Since cDNA clone inserts are generated by an essentially random process, there is one chance in three that the included cDNA will lie in the correct frame for proper translation. If the cDNA is not in the proper reading frame, it can be obtained by deletion or insertion of the appropriate number of bases by well known methods including <u>in vitro</u> mutagenesis, digestion with exonuclease III or mung bean nuclease, or oligonucleotide linker inclusion. FSEC will be expressed in the bacterial system as described.

Fsec cDNA can be shuttled into other vectors known to be useful for expression of protein in specific hosts. Oligonucleotide amplimers containing cloning sites as well as a segment of DNA sufficient to hybridize to stretches at both ends of the target cDNA (25 bases) can be synthesized chemically by standard methods. These primers can then used to amplify the desired gene segments by PCR. The resulting new gene segments can be digested with appropriate restriction enzymes under standard conditions and isolated by gel electrophoresis. Alternately, similar gene segments can be produced by digestion of the cDNA with appropriate

restriction enzymes and filling in the missing gene segments with chemically synthesized oligonucleotides. Segments of the coding sequence from more than one gene can be ligated together and cloned in appropriate vectors to optimize expression of recombinant sequence.

Suitable expression hosts for such chimeric molecules include but are not limited to mammalian cells such as Chinese Hamster Ovary (CHO) and human 293 cells, insect cells such as Sf9 cells, yeast cells such as Saccharomyces cerevisiae, and bacteria such as E. coli. For each of these cell systems, a useful expression vector may also include an origin of replication to allow propagation in bacteria and a selectable marker such as the ß-lactamase antibiotic resistance gene to allow selection in bacteria. In addition, the vectors may include a second selectable marker such as the neomycin phosphotransferase gene to allow selection in transfected eukaryotic host cells. Vectors for use in eukaryotic expression hosts may require RNA processing elements such as 3' polyadenylation sequences if such are not part of the cDNA of interest.

Additionally, the vector may contain promoters or enhancers which increase gene expression. Such promoters are host specific and include MMTV, SV40, or metallothionine promoters for CHO cells; trp, lac, tac or T7 promoters for bacterial hosts, or alpha factor, alcohol oxidase or PGH promoters for yeast. Transcription enhancers, such as the rous sarcoma virus (RSV) enhancer, may be used in mammalian host cells. Once homogeneous cultures of recombinant cells are obtained through standard culture methods, large quantities of recombinantly produced FSEC can be recovered from the conditioned medium and analyzed using chromatographic methods known in the art.

In addition to recombinant production, FSEC fragments may be produced by direct peptide synthesis using solid-phase techniques (cf Stewart et al (1969) Solid-Phase Peptide Synthesis, WH Freeman Co, San Francisco; Merrifield J (1963) J Am Chem Soc 85:2149-2154. In vitro protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Foster City, California) in accordance with the instructions provided by the manufacturer. Various fragments of FSEC may be chemically synthesized separately and combined using chemical methods to produce the full length FSEC.

FSEC for use in the induction of antibodies must be immunogenic. Peptides for use in the induction of FSEC-specific antibodies will comprise an amino acid sequence consisting of at least five amino acids and preferably at least 10 amino acids such that the peptide retains the three-dimensional configuration of a portion of the naturally occurring FSEC and may contain the entire amino acid sequence of the naturally occurring FSEC. Short stretches of FSEC amino acid may be fused with those of another protein such as keyhole limpet hemocyanin and the chimeric molecule used for antibody production.

Various methods are known to those of skill in the art for preparing monoclonal and polyclonal antibodies to FSEC. In one approach, denatured FSEC from the reverse phase HPLC separation is obtained and used to immunize mice or rabbits using techniques known to those of skill in the art. About 100 micrograms are adequate for immunization of a mouse, while up to 1 mg might be used for immunization of a rabbit. For identifying mouse hybridomas, the denatured protein can be radioiodinated and used to screen potential murine B-cell hybridomas for those which produce antibody. This procedure requires only small quantities of protein, such that 20 mg would be sufficient for labeling and screening of several thousand clones.

In another approach, the amino acid sequence of FSEC, as deduced from translation of the cDNA sequence, is analyzed to determine regions of high immunogenicity. For example, oligopeptides comprising hydrophilic regions, as shown in Figure 3, can be synthesized and used in suitable immunization protocols to raise antibodies. Analysis to select appropriate epitopes is described by Ausubel FM et al (1989, Current Protocols in Molecular Biology, John Wiley & Sons, NYC). The optimal amino acid sequences for immunization are usually at the C-terminus, the N-terminus and those intervening, hydrophilic regions of the polypeptide which are likely to be exposed to the external environment when the protein is in its natural conformation.

Typically, selected peptides, about 15 residues in length, are synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry and coupled to keyhole limpet hemocyanin (KLH, Sigma) by reaction with M-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS; cf. Ausubel FM et al, supra). If necessary, a cysteine may be introduced at the N-terminus of the peptide to permit coupling to KLH and animals can be

immunized with the peptide-KLH complex in complete Freund's adjuvant. The resulting antisera can be tested for antipeptide activity by binding the peptide to plastic, blocking with 1% BSA, reacting with antisera, washing and reacting with labeled (radioactive or fluorescent), affinity purified, specific goat anti-rabbit IgG.

Hybridomas may also be prepared and screened using standard techniques. Hybridomas of interest can be detected by screening with labeled FSEC to identify those fusions producing the monoclonal antibody with the desired specificity. In a typical protocol, wells of plates (FAST; Becton-Dickinson, Palo Alto, CA) are coated with affinity purified, specific rabbit-anti-mouse (or suitable anti-species Ig) antibodies at 10 mg/ml. The coated wells are blocked with 1% BSA, washed and exposed to supernatants from hybridomas. After incubation, the wells are exposed to labeled FSEC at 1 mg/ml. Clones producing antibodies will bind a quantity of labeled FSEC which is detectable above background. Such clones can be expanded and subjected to 2 cycles of cloning at limiting dilution (1 cell/3 wells). Cloned hybridomas are injected into pristane-treated mice to produce ascites, and monoclonal antibody can be purified from mouse ascitic fluid by affinity chromatography using Protein A. Monoclonal antibodies with affinities of at least 10^{8} M^{-1}, preferably 10^{9} to 10^{10} or stronger, will typically be made by standard procedures as described in Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory New York; and in Goding (1986) Monoclonal Antibodies: Principles and Practice, Academic Press, New York City, both incorporated herein by reference.

Antibodies specific for FSEC may be produced by inoculation of an appropriate animal with the polypeptide or an antigenic fragment. An antibody is specific for FSEC if it is produced against all or part of FSEC and binds to all or part of FSEC. Induction of antibodies includes not only the stimulation of an immune response by injection into animals, but also analogous steps in the production of synthetic antibodies or other specific-binding molecules such as the screening of recombinant immunoglobulin libraries (Orlandi R et al (1989) PNAS 86:3833-3837, or Huse WD et al (1989) Science 256:1275-1281) or the <u>in vitro</u> stimulation of lymphocyte populations. Current technology (Winter G and Milstein C (1991) Nature 349:293-299) provides for a number of highly specific binding reagents based on the principles of antibody formation. These techniques

may be adapted to produce molecules specifically binding FSEC.

The present invention also relates to the use of nucleic acid sequences encoding FSEC and anti-FSEC antibodies to treat or ameliorate the symptoms of disease states or conditions associated with excess expression of FSEC, such as FSEC associated inflammation, such as in viral or bacterial infection; diseases affecting immunoregulation such as autoimmune diseases; AIDS associated inflammation; and inflammation associated with diseases of erythrocytes or blood flow; and mechanical injury associated with trauma. For example, an fsec anti-sense molecule can be administered to an individual subject to FSEC associated inflammation to inhibit the translation of endogenous fsec, thereby ameliorating the symptoms of inflammation. The present invention also relates to the use of FSEC or nucleic acid sequences encoding FSEC to treat or ameliorate the symptoms of disease states or conditions where the immune system is compromised, such as in AIDS. In such conditions; administration of FSEC or nucleic acid encoding FSEC would replace or augment naturally occurring FSEC and function to activate and attract other molecules of the immune system.

Antibodies, inhibitors, receptors or antagonists of FSEC (or other treatments for excessive FSEC production, hereinafter abbreviated TEC), can provide different effects when administered therapeutically. TECs will be formulated in a nontoxic, inert, pharmaceutically acceptable aqueous carrier medium preferably at a pH of about 5 to 8, more preferably 6 to 8, although the pH may vary according to the characteristics of the antibody, inhibitor, receptor or antagonist being formulated and the condition to be treated. Characteristics of TEC include solubility of the molecule, half-life and antigenicity/immunogenicity; these and other characteristics may aid in defining an effective carrier. Native human proteins are preferred as TECs, but organic or synthetic molecules resulting from drug screens may be equally effective in particular situations.

TECs may be delivered by known routes of administration including but not limited to topical creams and gels; transmucosal spray and aerosol, transdermal patch and bandage; injectable, intravenous and lavage formulations; and orally administered liquids and pills, particularly formulated to resist stomach acid and enzymes. The particular formulation, exact dosage, and route of administration will be determined by the attending physician and will vary according to each specific situation.

Such determinations are made by considering multiple variables such as the condition to be treated, the TEC to be administered, and the pharmacokinetic profile of the particular TEC. Additional factors which may be taken into account include disease state (e.g. severity) of the patient, age, weight, gender, diet, time of administration, drug combination, reaction sensitivities, and tolerance/response to therapy. Long acting TEC formulations might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular TEC.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see USPN 4,657,760; 5,206,344; or 5,225,212. It is anticipated that different formulations will be effective for different TECs and that administration targeting the spleen may necessitate delivery in a manner different from that to another organ or tissue.

It is contemplated that conditions or diseases of the spleen or other tissues which activate monocytes, macrophages, basophils, eosinophils or other leukocytes may precipitate damage that is treatable with TECs. These conditions or diseases may be specifically diagnosed by the tests discussed above, and such testing should be performed in suspected cases of inflammation or viral or bacterial infections including mononucleosis, and malaria; AIDS associated inflammation; mechanical injury associated with trauma; diseases affecting immunoregulation such as rheumatoid arthritis or systemic lupus erythematosis; and diseases of erythrocytes or blood flow including sickle cell anemia and β thalassemia.

The examples below are provided to illustrate the subject invention. These examples are provided by way of illustration and are not included for the purpose of limiting the invention.

EXAMPLES

I Isolation of mRNA and construction of cDNA libraries

The fsec cDNA sequence was identified among the sequences comprising a human fetal spleen library. This library was constructed from

pooled human fetal spleen tissues by Stratagene Inc. (Cat. #937205; 11011 N Torrey Pines Rd, La Jolla, CA 92037). cDNA synthesis was primed using oligo dT hexamers, and synthetic adapter oligonucleotides were ligated onto cDNA ends enabling its insertion into the UNI-ZAP™ vector system (Stratagene Inc). This vector allows high efficiency unidirectional (sense orientation) lambda library construction and the convenience of a plasmid system with blue/white color selection to detect clones with cDNA insertions.

The quality of the each cDNA library was screened using either DNA probes or antibody probes, and then the pBluescript® phagemid (Stratagene Inc) was rapidly excised in living cells. The phagemid allows the use of a plasmid system for easy insert characterization, sequencing, site-directed mutagenesis, the creation of unidirectional deletions and expression of fusion proteins. Phage particles from each library were infected into the E.coli host strain XL1-BLUE® (Stratagene Inc). The high transformation efficiency of XL1-BLUE increases the probability of obtaining rare, under-represented clones from the cDNA library.

II Isolation of cDNA Clones

The phagemid forms of individual cDNA clones were obtained by the in vivo excision process, in which XL1-BLUE was coinfected with an f1 helper phage. Proteins derived from both lambda phage and f1 helper phage initiated new DNA synthesis from defined sequences on the lambda target DNA and create a smaller, single-stranded circular phagemid DNA molecule that includes all DNA sequences of the pBluescript plasmid and the cDNA insert. The phagemid DNA was released from the cells and purified, then used to re-infect fresh bacterial host cells (SOLR, Stratagene Inc), where the double-stranded phagemid DNA was produced. Because the phagemid carries the gene for ß-lactamase, the newly transformed bacteria were selected on medium containing ampicillin.

Phagemid DNA was purified using the QIAWELL-8 Plasmid Purification System from QIAGEN® DNA Purification System (QIAGEN Inc, 9259 Eton Ave, Chatsworth, CA 91311). This technique provides a rapid and reliable high-throughput method for lysing the bacterial cells and isolating highly purified phagemid DNA. The DNA eluted from the purification resin was suitable for DNA sequencing and other analytical manipulations.

III **Sequencing of cDNA Clones**

The cDNA inserts from random isolates of the human fetal spleen library were sequenced in part. The cDNAs were sequenced by the method of Sanger F. and AR Coulson (1975; J. Mol. Biol.94:441f), using a Hamilton Micro Lab 2200 (Hamilton, Reno NV) in combination with four Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown MA) and Applied Biosystems 377 or 373 DNA Sequencing Systems (Perkin Elmer) and reading frame determined.

IV **Homology Searching of cDNA Clones and Deduced Protein**

Each cDNA was compared to sequences in GenBank using a search algorithm developed by Applied Biosystems and incorporated into the INHERIT™ 670 Sequence Analysis System. In this algorithm, Pattern Specification Language (developed by TRW Inc.) was used to determine regions of homology. The three parameters that determine how the sequence comparisons run were window size, window offset, and error tolerance. Using a combination of these three parameters, the DNA database was searched for sequences containing regions of homology to the query sequence, and the appropriate sequences were scored with an initial value. Subsequently, these homologous regions were examined using dot matrix homology plots to distinguish regions of homology from chance matches. Smith-Waterman alignments were used to display the results of the homology search.

Peptide and protein sequence homologies were ascertained using the INHERIT 670 Sequence Analysis System in a way similar to that used in DNA sequence homologies. Pattern Specification Language and parameter windows were used to search protein databases for sequences containing regions of homology which were scored with an initial value. Dot-matrix homology plots were examined to distinguish regions of significant homology from chance matches.

BLAST, which stands for Basic Local Alignment Search Tool (Altschul SF (1993) J Mol Evol 36:290-300; Altschul, SF et al (1990) J Mol Biol 215:403-10), was used to search for local sequence alignments . BLAST produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologs. BLAST is useful for matches which do not contain gaps. The

fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP).

An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user. The BLAST approach is to look for HSPs between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search. Any database sequence whose match satisfies E is reported in the program output.

The nucleotide and amino acid sequences for the fetal spleen expressed chemokine, FSEC, are shown in Figure 1 (SEQ ID NO:1 and SEQ ID NO:2, respectively. Inherit analysis revealed that Incyte clone 29592.db (see Figure 2), contained a significant portion of the nucleotide sequence depicted in SEQ ID NO:1 which encodes FSEC. The 5' nucleotide sequence encoding FSEC was obtained using PCR technology. In the first round of amplification, one oligomer specific to a portion of the chemokine 29592.db, TCC TTC CTT CTG GTC CTC GGT TCC, and another specific to the vector containing the fetal spleen cDNA inserts, GGA AAC AGC TAT GAC CAT G, were used to identify and amplify the 5' fsec nucleotide sequence. In the second round of amplification, a set of nested oligomers i.e., one internal to the previous 5' oligomer, consisting of the nucleotides, CTT GGA ATT CAC TCC GGG CTC CCT CTG CAC G, and the primer specific to the vector sequence (shown above) were used to further amplify the cDNA. The missing 5' sequence was isolated using ECO RI (which digests the DNA within the underlined restriction site in the internal oligomer above), subjected to electrophoresis and ligated to the 3' portion of the chemokine sequence from 29592.db. The full length molecule designated 29592 was resequenced.

V Identification and Full Length Sequencing of the Gene

The fsec nucleotide sequence is homologous to but clearly different from any known C-C chemokine molecule. The complete nucleotide sequence for fsec is shown as Incyte clone 29592. When all three possible

predicted translations of the sequence were searched against protein databases such as SwissProt and PIR. no exact matches were found to the possible translations of fsec. Figure 2 shows the comparison of FSEC with other C-C chemokines. The regions of homology among the chemokines, including the definitive C-C motif, are shaded. The phylogenetic analysis (Figure 4) shows how closely FSEC is related to other well characterized human C-C chemokines. The most related of these molecules cluster together at the right hand side of the figure.

VI Antisense analysis

Knowledge of the correct, complete cDNA sequences of novel expressed chemokine genes will enable their use in antisense technology in the investigation of gene function. Either oligonucleotides, genomic or cDNA fragments comprising the antisense strand of fsec can be used either in vitro or in vivo to inhibit expression of the specific protein. Such technology is now well known in the art, and probes can be designed at various locations along the nucleotide sequence. By treatment of cells or whole test animals with such antisense sequences, the gene of interest can be effectively turned off. Frequently, the function of the gene can be ascertained by observing behavior at the cellular, tissue or organismal level (e.g. lethality, loss of differentiated function, changes in morphology, etc.).

In addition to using sequences constructed to interrupt transcription of the open reading frame, modifications of gene expression can be obtained by designing antisense sequences to intron regions, promoter/enhancer elements, or even to trans-acting regulatory genes. Similarly, inhibition can be achieved using Hogeboom base-pairing methodology, also known as "triple helix" base pairing.

VII Expression of FSEC

The nucleotide sequences encoding FSEC were cloned into an expression vector that comprises a T7 promoter followed by an initiating methionine codon (ATG), followed by six histidine codons, followed by the TrxA gene of E.coli (which encodes thioredoxin), followed by a sequence coding for an enterokinase cleavage site and nucleotide sequences encoding FSEC. Empirical studies associated with cleavage of signal sequences

indicate that cleavage occurs at or near the C-terminal end of a predicted hydrophobic region located at the N-terminus of the full length protein. The hydrophobicity profile of FSEC is shown in Figure 3 and based on this profile, residue 16 (proline) of SEQ ID NO:2, was chosen as the N-terminal amino acid residue for expression of mature FSEC.

To determine the naturally processed N-terminal amino acid residue of mature FSEC, the nucleotide sequence disclosed in SEQ ID NO:1 is ligated into an expression vector appropriate for eukaryotic expression. The expression vector comprising SEQ ID NO:1 is transfected into an appropriate host cell and the transfected host cell is subjected to conditions appropriate for expression of the protein. The amino acid sequence of the processed mature protein is confirmed through N-terminal protein sequence analysis techniques known to those of skill in the art.

The expression vectors described above containing the 6 histidine codons were used to transform a host cell, the host cell culture was induced with IPTG and the expressed protein was subjected to denaturing SDS poly acrylamide gel electrophoresis. Nucleic acid from the expression vector was partially purified using the miniprep procedure of Sambrook supra which produced super-coiled DNA. About 100 ng of DNA were used to transform the host bacterial cell, W3110/DE3. W3110/DE3 was constructed using W3110 from the ATCC and the lambda DE3 lysogenization kit commercially available from Novagen. DE3 lysogens are often less competent than their parent, W3110, and are adapted to use super-coiled DNA for efficient transformation.

A single transformant from the FSEC transformation was selected and used to inoculate a 5 ml culture of L-broth containing ampicillin. Each 5 ml culture was grown overnight (12-15 hours) at 37 degrees C. with shaking. The next day, 1 ml of the overnight culture was used to inoculate a 100 ml culture of L-broth with ampicillin in a 500 ml flask and allowed to grow at 37 degrees C. with shaking until the OD600 of the culture reached 0.4-0.6. If inoculated cells are allowed to grow past an OD600 of 0.6, they will begin to reach stationary phase and induction levels will be reduced.

At the time of inoculation, a 5 ml sample was removed, placed on ice and used as a pre-induction (or 0 hour) sample. When the cell culture reached an OD600 of 0.6, 400µl of an 100mM IPTG stock solution was added for a final concentration of 0.4mM. The cultures were allowed to

grow for 3 hours at 37 degrees C. with shaking. Analysis of induction was determined by sampling 5 ml aliquots of the culture at 1 hour intervals up to 6 hours and analysing on a denaturing SDS poly acrylamide gel electrophoresis. The fusion protein appeared to accumulate in both the soluble and insoluble fraction of the cells.

Maximal induction of FSEC occurred by 2 hours. Growth beyond 4 hours resulted in lysis in the culture and overall reduced yields of the desired protein due to proteolysis. Five ml aliquots of the cell cultures were obtained at 0, and 2 hours and centrifuged for 5 minutes at 3000 RPM at 4 degrees C. The supernatant was aspirated and the pellets were subjected to a freeze-thaw step to help lyse the cells. The pellet was resuspended in TE [10mM Tris-HCL pH 8.0, 1mM EDTA pH 8.0] at 4 degrees C. at a volume calculated as: vol TE(ul) = (OD600)(250), and an equivalent volume of 2X SDS Sample Loading Buffer (Novex) was added to each sample. The samples were boiled for 5 minutes and 10ul of each sample was loaded per lane.

The expected molecular weight of the fusion protein comprising FSEC is 23,145 Daltons. Analysis of the expressed FSEC on a 14% SDS-polyacrylamide gel shows an apparent molecular weight of about 32kDa. N-terminal protein sequence analysis of this band yielded a string of histidine residues which is consistent with the predicted sequence. A second fusion protein lacking the six histidine residues was constructed and expressed. This fusion protein also had an apparent molecular weight greater than its predicted value. N-terminal protein sequence analysis of this product matched the sequence for thioredoxin which is consistent with the expected results.

VIII Isolation of Recombinant FSEC

FSEC was expressed as a chimeric protein having six histidines followed by thioredoxin (TrxA of E.coli) with an enterokinase cleavage site between the TrxA protein and FSEC. The histidines were added to facilitate protein purification. The presence of the histidines allows for purification on IMIAC chromatography (Porath supra).

IX Diagnostic Test Using FSEC-Specific Antibodies

Particular FSEC antibodies are useful for the diagnosis of

prepathologic conditions, and chronic or acute diseases which are characterized by differences in the amount or distribution of FSEC. FSEC is likely to be specific for abnormalities or pathologies of tissues where it is found.

Diagnostic tests for FSEC include methods utilizing an anti-FSEC antibody and a label to detect FSEC in human body fluids, tissues or extracts of such tissues. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, the polypeptides and antibodies will be labeled by joining them, either covalently or noncovalently, with a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and have been reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. Patents teaching the use of such labels include USPNs 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced as shown in USPN 4,816,567, incorporated herein by reference.

A variety of protocols for measuring soluble or membrane-bound FSEC, using either polyclonal or monoclonal antibodies specific for that FSEC are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on FSEC is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, DE et al (1983, J Exp Med 158:1211).

X Purification of Native FSEC Using Specific Antibodies

Native or recombinant FSEC is purified by immunoaffinity chromatography using antibodies specific for FSEC. An immunoaffinity column is constructed by covalently coupling the anti-FSEC antibody to an activated chromatographic resin.

Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia LKB Biotechnology, Piscataway, NJ).

Alternatively, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated Sepharose (Pharmacia LKB Biotechnology). The antibody is coupled to the resin, the resin is blocked, and the derivative resin is washed according to the manufacturer's instructions.

Such an immunoaffinity column is utilized in the purification of FSEC by preparing a fraction from cells containing FSEC in a soluble form. This preparation is derived by solubilization of the whole cell or of a subcellular fraction obtained via differential centrifugation by the addition of detergent or by other methods well known in the art. Alternatively, soluble FSEC containing a signal sequence is secreted in useful quantity into the medium in which the cells are grown.

A soluble FSEC-containing preparation is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of FSEC (eg, high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/FSEC binding (e.g., a buffer of pH 2-3 or a high concentration of a chaotrope such as urea or thiocyanate ion), and FSEC is collected.

XI **Determination of FSEC-Induced Chemotaxis or Cell Activation**

The chemotactic activity of FSEC is measured in a 48-well microchemotaxis chamber (Falk WR et al (1980) J Immunol Methods 33:239). In each well, two compartments are separated by a filter that allows the passage of cells in response to a chemical gradient. Cell culture medium such as RPMI 1640 (Sigma, St. Louis MO) containing FSEC is placed on one side of a filter, usually polycarbonate, and cells suspended in the same media are placed on the opposite side of the filter. Sufficient incubation time is allowed for the cells to traverse the filter in response to the concentration gradient across the filter. Filters are recovered from each well, and cells adhering to the side of the filter facing FSEC are typed and quantified.

The specificity of the chemoattraction is determined by performing the chemotaxis assay on specific populations of cells. First, blood cells obtained from venipuncture are fractionated by density gradient

centrifugation and the chemotactic activity of FSEC is tested on enriched populations of neutrophils, peripheral blood mononuclear cells, monocytes and lymphocytes. Optionally, such enriched cell populations are further fractionated using $CD8^+$ and $CD4^+$ specific antibodies for negative selection of $CD4^+$ and $CD8^+$ enriched T-cell populations, respectively.

Another assay elucidates the chemotactic effect of FSEC on activated T-cells. There, unfractionated T-cells or fractionated T-cell subsets are cultured for 6 to 8 hours in tissue culture vessels coated with CD-3 antibody. After this CD-3 activation, the chemotactic activity of FSEC is tested as described above. Many other methods for obtaining enriched cell populations are known in the art.

Some chemokines also produce a non-chemotactic cell activation of neutrophils and monocytes. This is tested via standard measures of neutrophil activation such as actin polymerization, increase in respiratory burst activity, degranulation of the azurophilic granule and mobilization of Ca^{++} as part of the signal transduction pathway. The assay for mobilization of Ca^{++} involves preloading neutrophils with a fluorescent probe whose emission characteristics have been altered by Ca^{++} binding. When the cells are exposed to an activating stimulus, Ca^{++} flux is determined by observation of the cells in a fluorometer. The measurement of Ca^{++} mobilization has been described in Grynkievicz G et al. (1985) J Biol Chem 260:3440, and McColl S et al. (1993) J Immunol 150:4550-4555, incorporated herein by reference.

Degranulation and respiratory burst responses are also measured in monocytes (Zachariae COC et al. (1990) J Exp Med 171: 2177-82). Further measures of monocyte activation are regulation of adhesion molecule expression and cytokine production (Jiang Y et al. (1992) J Immunol 148: 2423-8). Expression of adhesion molecules also varies with lymphocyte activation (Taub D et al. (1993) Science 260: 355-358).

XII Drug Screening

FSEC, or biologically or immunologically fragments thereof, are used for screening compounds in any of a variety of drug screening techniques.

The FSEC, or a fragment thereof, that is employed in such a test is either free in solution, affixed to a solid support, borne on a cell surface or

located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which have been stably transformed with recombinant nucleic acids expressing the FSEC or a fragment thereof. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, are used in standard binding assays. One may measure, for example, the formation of a complex between FSEC, or a fragment thereof, and the agent being tested. Alternatively, the diminution in complex formation between FSEC and its target cell, monocyte, etc. caused by the agent being tested is monitored.

Thus, the present invention provides methods of screening for drugs or any other agents which can affect inflammation and disease. These methods comprise contacting a drug or agent to be tested with an FSEC polypeptide or fragment thereof and assaying (i) for the presence of a complex between the agent and the FSEC polypeptide or fragment, or (ii) for the presence of a complex between the FSEC polypeptide or fragment and the cell, by methods well known in the art. In such competitive binding assays, the FSEC polypeptide or fragment is labeled. After suitable incubation, free FSEC polypeptide or fragment is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the particular agent to bind to FSEC or to interfere with the FSEC/cell complex.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the FSEC polypeptides and is described in detail in EPA 84/03564, published on September 13, 1984, incorporated herein by reference. Briefly stated, a plurality of different peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with FSEC polypeptide and washed. Bound FSEC polypeptide is then detected by methods well known in the art. Purified FSEC can also be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies can be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding FSEC specifically compete with a test compound for binding to FSEC polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the

presence of any peptide which shares one or more antigenic determinants with FSEC.

XIII Rational Drug Design

The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact, e.g., agonists, antagonists, or inhibitors. Any of these examples can be used to fashion drugs which are more active or stable forms of the polypeptide or which enhance or interfere with the function of a polypeptide in vivo (cf Hodgson J (1991) Bio/Technology 9:19-21, incorporated herein by reference).

In one approach, the three-dimensional structure of a protein of interest, or of a protein-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Useful information regarding the structure of a polypeptide may be gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous chemokine-like molecules or to identify efficient inhibitors. Useful examples of rational drug design may include molecules which have improved activity or stability as shown by Braxton S and Wells JA (1992 Biochemistry 31:7796-7801) or which act as inhibitors, agonists, or antagonists of naturally occurring peptides as shown by Athauda SB et al (1993 J Biochem 113:742-746), incorporated herein by reference.

It is also possible to isolate a target-specific antibody, selected by functional assay, as described above, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides would then act as the pharmacore.

Using methods described infra, a sufficient amount of FSEC

may be made available to perform analytical studies as X-ray crystallography. In addition, knowledge of the FSEC amino acid sequence disclosed herein will provide guidance to those employing computer modeling techniques in place of or in addition to x-ray crystallography.

XIV Identification of FSEC Receptors

Purified FSEC, or a fragment thereof, can be used to characterize and purify specific cell surface receptors and other binding molecules. Cells which respond to FSEC by chemotaxis or other specific responses are likely to express a receptor for FSEC. Radioactive labels are incorporated into FSEC, or a fragment thereof, by various methods known in the art. A preferred embodiment is the labeling of primary amino groups in FSEC with ^{125}I Bolton-Hunter reagent (Bolton, AE and Hunter, WM (1973) Biochem J 133: 529), which has been used to label other chemokines without concomitant loss of biological activity (Hebert CA et al (1991) J Biol Chem 266: 18989; McColl S et al (1993) J Immunol 150:4550-4555). Receptor-bearing cells are incubated with labeled FSEC. The cells are then washed to removed unbound FSEC, and receptor-bound FSEC is quantified. The data obtained using different concentrations of FSEC are used to calculate values for the number and affinity of receptors.

Labeled FSEC is useful as a reagent for purification of its specific receptor. In one embodiment of affinity purification, FSEC is covalently coupled to a chromatography column. Receptor-bearing cells are extracted, and the extract is passed over the column. The receptor binds to the column by virtue of its biological affinity for FSEC. The receptor is recovered from the column and subjected to N-terminal protein sequencing. This amino acid sequence is then used to design degenerate oligonucleotide probes for cloning the receptor gene.

In an alternate method, mRNA is obtained from receptor-bearing cells and made into a cDNA library. The library is transfected into a population of cells, and those cells expressing the receptor are selected using fluorescently labeled FSEC. The receptor is identified by recovering and sequencing recombinant DNA from highly labeled cells.

In another alternate method antibodies, preferably monoclonal antibodies, are raised against the surface of receptor-bearing cells. The

monoclonal antibodies are screened to identify those which inhibit the binding of labeled FSEC. These monoclonal antibodies are then used in affinity purification or expression cloning of the receptor.

Soluble receptors or other soluble binding molecules are identified in a similar manner. Labeled FSEC is incubated with extracts or other appropriate materials derived from the spleen. After incubation, FSEC complexes larger than the size of the purified FSEC are identified by a sizing technique such as, for example, size exclusion chromatography or density gradient centrifugation, and are purified by methods known in the art. The soluble receptors or binding protein(s) are subjected to N-terminal sequencing to obtain information sufficient for database identification, if the soluble protein is known, or for cloning, if the soluble protein is unknown.

All publications and patents mentioned in the above specification are herein incorporated by reference. The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. Indeed, various modifications of the above described modes for carrying out the invention which are obvious to those skilled in the field of molecular biology or related fields are intended to be within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Incyte Pharmaceuticals, Inc.

(ii) TITLE OF THE INVENTION: A NEW CHEMOKINE EXPRESSED IN FETAL SPLEEN, ITS PRODUCTION AND USES

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Incyte Pharmaceuticals, Inc.
(B) STREET: 3174 Porter Drive
(C) CITY: Palo Alto
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 94304

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 1.5

(vi) CURRENT APPLICATION DATA:
(A) PCT APPLICATION NUMBER: To Be Assigned
(B) FILING DATE: 19-JAN-1996
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/375,346
(B) FILING DATE: 19-JAN-1995

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Luther, Barbara J
(B) REGISTRATION NUMBER: 33,954
(C) REFERENCE/DOCKET NUMBER: PF-0026 PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 415-855-0555
(B) TELEFAX: 415-852-0195

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 719 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: HUMAN FETAL SPLEEN
(B) CLONE: 29592

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCAGGGCTCA CATTCCCAGC CTCACATCAC TCACACCTTG CATTTCACCC CTGCATCCCA 60
GTCGCCCTGC AGCCTCACAC AGATCCTGCA CACACCCAGA CAGCTGGCGC TCACACATTC 120

```
ACCGTTGGCC TGCCTCTGTT CACCCTCCAT GGCCCTGCTA CTGGCCCTCA GCCTGCTGGT    180
TCTCTGGACT TCCCCAGCCC CAACTCTGAG TGGCACCAAT GATGCTGAAG ACTGCTGCCT    240
GTCTGTGACC CAGAAACCCA TCCCTGGGTA CATCGTGAGG AACTTCCACT ACCTTCTCAT    300
CAAGGATGGC TGCAGGGTGC CTGCTGTAGT GTTCACCACA CTGAGGGGCC GCCAGCTCTG    360
TGCACCCCCA GACCAGCCCT GGGTAGAACG CATCATCCAG AGACTGCAGA GGACCTCAGC    420
CAAGATGAAG CGCCGCAGCA GTTAACCTAT GACCGTGCAG AGGGAGCCCG GAGTCCGAGT    480
CAAGCATTGT GAATTATTAC CTAACCTGGG GAACCGAGGA CCAGAAGGAA GGACCAGGCT    540
TCCAGCTCCT CTGCACCAGA CCTGACCAGC CAGGACAGGG CCTGGGGTGT GTGTGAGTGT    600
GAGTGTGAGC GAGAGGGTGA GTGTGGTCAG AGTAAAGCTG CTCCACCCCC AGATTGCAAT    660
GCTACCAATA AAGCCGCCTG GTGTTTACAA CTAAAAAAAA AAAAAAAAAA AAAAAAAAA     719
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: HUMAN FETAL SPLEEN
(B) CLONE: 29592

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ala Leu Leu Leu Ala Leu Ser Leu Leu Val Leu Trp Thr Ser Pro
 1               5                  10                  15
Ala Pro Thr Leu Ser Gly Thr Asn Asp Ala Glu Asp Cys Cys Leu Ser
            20                  25                  30
Val Thr Gln Lys Pro Ile Pro Gly Tyr Ile Val Arg Asn Phe His Tyr
        35                  40                  45
Leu Leu Ile Lys Asp Gly Cys Arg Val Pro Ala Val Val Phe Thr Thr
    50                  55                  60
Leu Arg Gly Arg Gln Leu Cys Ala Pro Pro Asp Gln Pro Trp Val Glu
65                  70                  75                  80
Arg Ile Ile Gln Arg Leu Gln Arg Thr Ser Ala Lys Met Lys Arg Arg
                85                  90                  95
Ser Ser
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 92 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala
 1               5                  10                  15
Leu Cys Asn Gln Phe Ser Ala Ser Leu Ala Ala Asp Thr Pro Thr Ala
            20                  25                  30
Cys Cys Phe Ser Tyr Thr Ser Arg Gln Ile Pro Gln Asn Phe Ile Ala
        35                  40                  45
Asp Tyr Phe Glu Thr Ser Ser Gln Cys Ser Lys Pro Gly Val Ile Phe
    50                  55                  60
```

```
Leu Thr Lys Arg Ser Arg Gln Val Cys Ala Asp Pro Ser Glu Glu Trp
65                  70                  75                  80
Val Gln Lys Tyr Val Ser Asp Leu Glu Leu Ser Ala
                85                  90
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 92 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Lys Leu Cys Val Thr Val Leu Ser Leu Leu Met Leu Val Ala Ala
 1               5                  10                  15
Phe Cys Ser Pro Ala Leu Ser Ala Pro Met Gly Ser Asp Pro Pro Thr
            20                  25                  30
Ala Cys Cys Phe Ser Tyr Thr Ala Arg Lys Leu Pro Arg Asn Phe Val
        35                  40                  45
Val Asp Tyr Tyr Glu Thr Ser Ser Leu Cys Ser Gln Pro Ala Val Val
    50                  55                  60
Phe Gln Thr Lys Arg Ser Lys Gln Val Cys Ala Asp Pro Ser Glu Ser
65                  70                  75                  80
Trp Val Gln Glu Tyr Val Tyr Asp Leu Glu Leu Asn
                85                  90
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 91 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Lys Val Ser Ala Ala Arg Leu Ala Val Ile Leu Ile Ala Thr Ala
 1               5                  10                  15
Leu Cys Ala Pro Ala Ser Ala Ser Pro Tyr Ser Ser Asp Thr Thr Pro
            20                  25                  30
Cys Cys Phe Ala Tyr Ile Ala Arg Pro Leu Pro Arg Ala His Ile Lys
        35                  40                  45
Glu Tyr Phe Tyr Thr Ser Gly Lys Cys Ser Asn Pro Ala Val Val Phe
    50                  55                  60
Val Thr Arg Lys Asn Arg Gln Val Cys Ala Asn Pro Glu Lys Lys Trp
65                  70                  75                  80
Val Arg Glu Tyr Ile Asn Ser Leu Glu Met Ser
                85                  90
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 78 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Gly Leu Ala Ala Ala Leu Leu Val Leu Val Cys Thr Met Ala Leu Cys
 1               5                  10                  15
Ser Cys Ala Gln Val Gly Thr Asn Lys Glu Leu Cys Cys Leu Val Tyr
            20                  25                  30
Thr Ser Trp Gln Ile Pro Gln Lys Phe Ile Val Asp Tyr Ser Glu Thr
        35                  40                  45
Ser Pro Gln Cys Pro Lys Pro Gly Val Ile Leu Leu Thr Lys Arg Gly
    50                  55                  60
Arg Gln Ile Cys Ala Asp Pro Asn Lys Lys Trp Val Gln Lys
65                  70                  75
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCCTTCCTTC TGGTCCTCGG TTCC 24

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGAAACAGCT ATGACCATG 19

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTTGGAATTC ACTCCGGGCT CCCTCTGCAC G 31

CLAIMS

1. A purified polynucleotide comprising a nucleic acid sequence encoding the polypeptide having the sequence as depicted in SEQ ID NO:2, or its complement.

2. The polynucleotide of Claim 1 wherein the nucleic acid sequence consists of SEQ ID NO:1.

3. An expression vector comprising the polynucleotide of Claim 2.

4. A host cell comprising the expression vector of Claim 3.

5. A nucleic acid probe comprising a non-conserved fragment of the polynucleotide of Claim 2.

6. An antisense molecule comprising a polynucleotide sequence complementary to at least a portion of the polynucleotide of Claim 2.

7. A method for producing a polypeptide comprising the sequence as depicted in SEQ ID NO:2, said method comprising:

a) culturing the host cells of Claim 4 under conditions suitable for the expression of the polypeptide, and

b) recovering said polypeptide from the cell culture.

8. A purified Fetal Spleen Expressed Chemokine having the amino acid sequence as depicted in SEQ ID NO:2.

9. A purified Fetal Spleen Expressed Chemokine having the N-terminal amino acid residue of residue 16, Proline, of SEQ ID NO:2..

10. An antibody specific for the purified polypeptide of Claim 9.

11. A diagnostic composition for the detection of nucleic acid sequences encoding Fetal Spleen Expressed Chemokine (FSEC) comprising the nucleic acid

probe of Claim 5.

12. A diagnostic test for the detection of nucleotide sequences encoding Fetal Spleen Expressed Chemokine (FSEC) in a biological sample, comprising the steps of:

a) combining the biological sample with a first nucleotide which comprises the nucleotide sequence of SEQ ID NO:1 or a fragment thereof, wherein said fragment is derived from a non-conserved region of said nucleotide, under conditions suitable for the formation of a nucleic acid hybridization complex;

b) detecting said hybridization complex wherein the presence of said complex correlates with the presence of a second nucleotide encoding FSEC in said biological sample; and

c) comparing the amount of the second nucleotide in said sample with a standard thereby determining whether the amount of said second nucleotide varies from the standard, wherein the presence of an abnormal level of said second nucleotide correlates positively with a condition associated with aberrant expression of FSEC.

13. The diagnostic test of Claim 12 wherein said first nucleotide is labeled with a reporter molecule and the hybridization complex is detected by measuring said reporter molecule.

14. A diagnostic test for the detection of nucleotide sequences encoding Fetal Spleen Expressed Chemokine (FSEC) in a biological sample, comprising the steps of:

a) combining the biological sample with polymerase chain reaction primers under conditions suitable for nucleic acid amplification, wherein said primers comprise fragments from non-conserved regions of the nucleotide sequence of SEQ ID NO:1;

b) detecting amplified nucleotide sequences; and

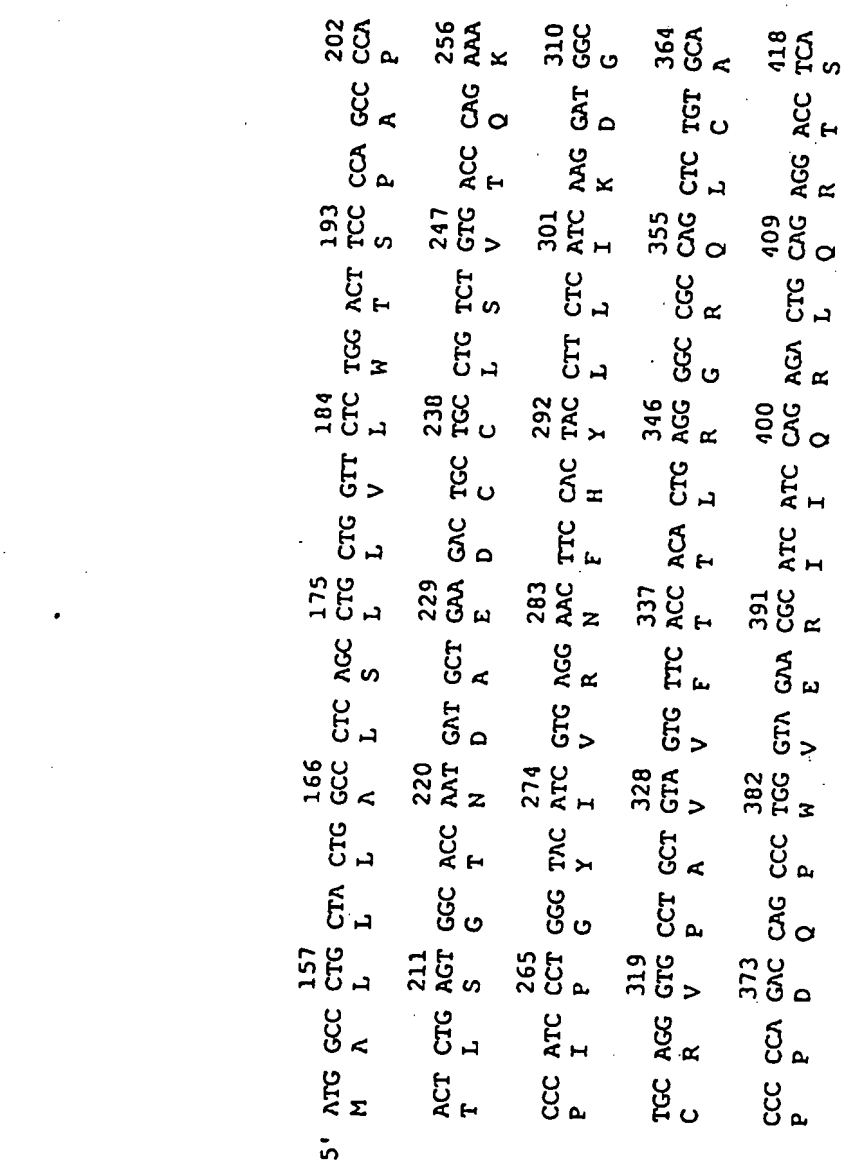
c) comparing the amount of amplified nucleotide sequences in said biological sample with a standard thereby determining whether the amount of said nucleotide sequence varies from said standard, wherein the presence of an abnormal level of said nucleotide sequence correlates positively with a condition associated with aberrant expression of FSEC.

15. A method of screening a plurality of compounds for specific binding affinity with the polypeptide of Claim 8 or any portion thereof, comprising the steps of:

a) providing a plurality of compounds;

b) combining Fetal Spleen Expressed Chemokine (FSEC) with each of a plurality of compounds for a time sufficient to allow binding under suitable conditions; and

c) detecting binding of FSEC to each of the plurality of compounds, thereby identifying the compounds which specifically bind FSEC.


5' ATG GCC CTG CTA CTG GCC CTC AGC CTG CTG GTT CTC TGG ACT TCC CCA GCC CCA
M A L L L A L S L L V L W T S P A P
157 166 175 184 193 202
ACT CTG AGT GGC ACC AAT GAT GCT GAA GAC TGC CTG TCT GTG ACC CAG AAA
T L S G T N D A E D C L S V T Q K
211 220 229 238 247 256
CCC ATC CCT GGG TAC ATC AGG AAC TTC CAC TAC CTT CTC ATC AAG GAT GGC
P I P G Y I R N F H Y L L I K D G
265 274 283 292 301 310
TGC AGG GTG CCT GCT GTA GTG TTC ACC ACA CTG AGG GGC CGC CAG CTC TGT GCA
C R V P A V V F T T L R G R Q L C A
319 328 337 346 355 364
CCC CCA GAC CAG CCC TGG GTA GAA CGC ATC ATC CAG AGA CTG CAG AGG ACC TCA
P P D Q P W V E R I I Q R L Q R T S
373 382 391 400 409 418
GCC AAG ATG AAG CGC CGC AGC AGT TAA 3'
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427 436 445

FIGURE 1

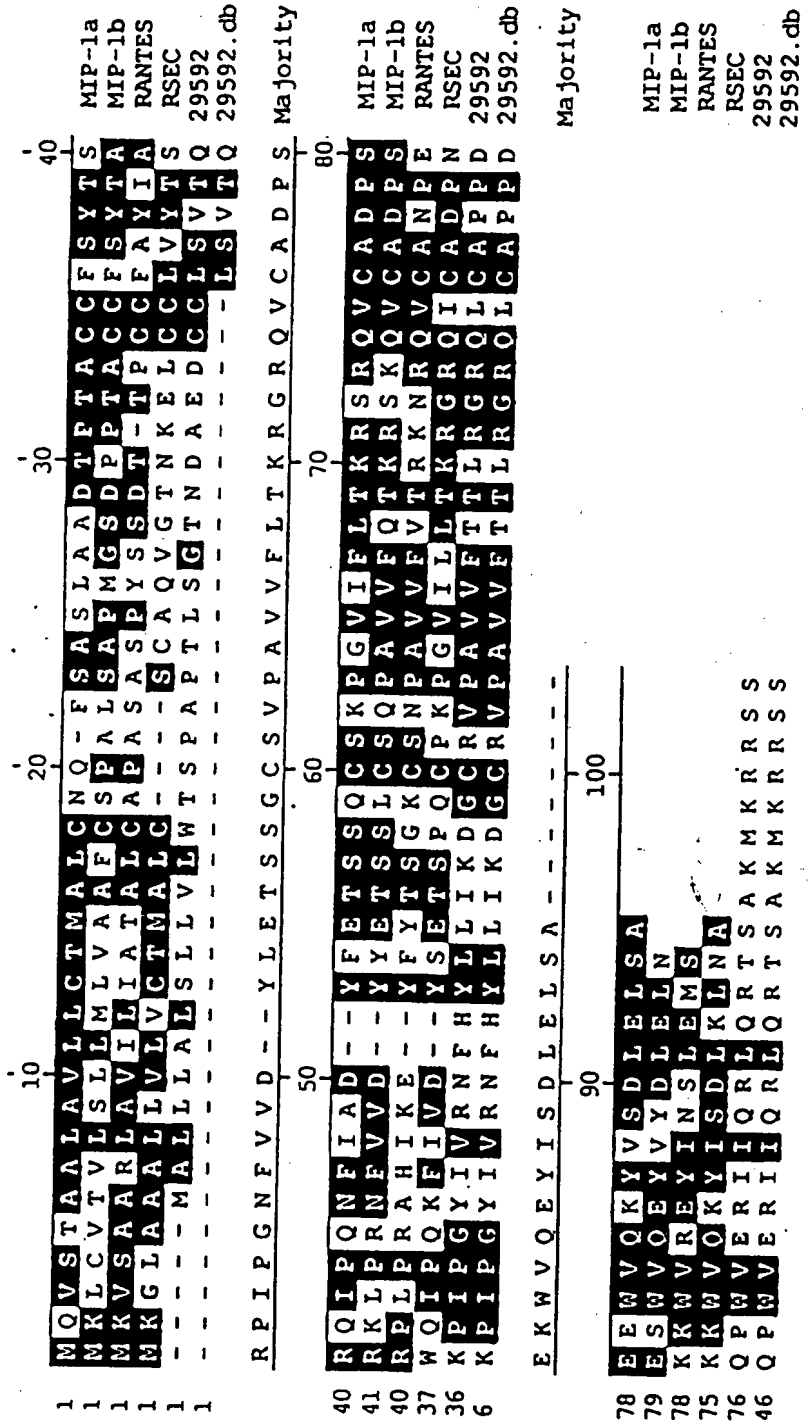

FIGURE 2

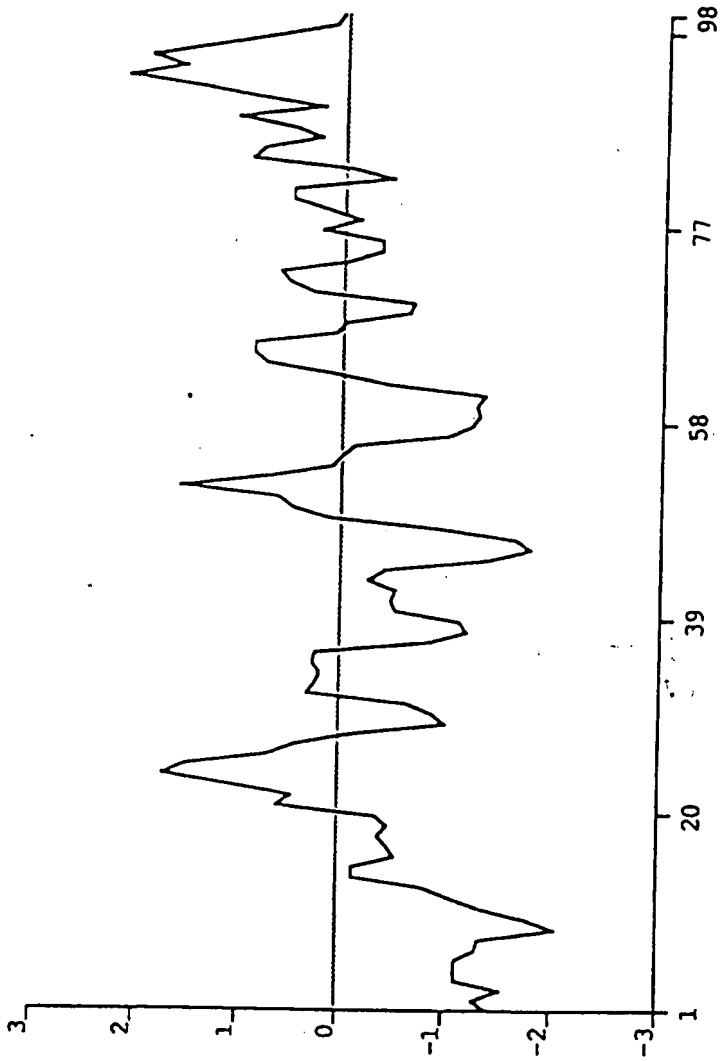

FIGURE 3

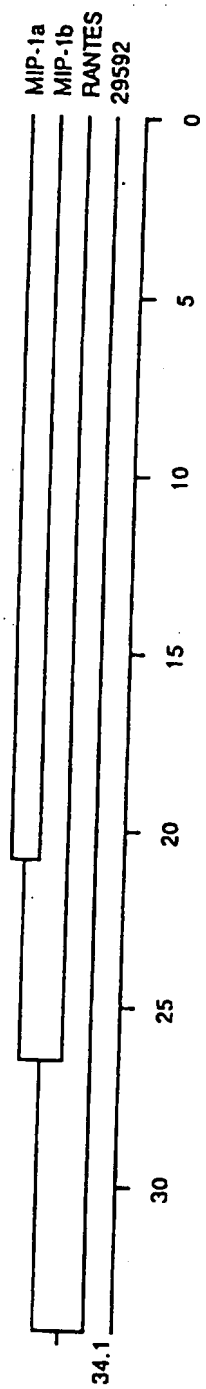

FIGURE 4

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/US 96/00920

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/19 C07K14/52 C12N1/21 C07K16/24 C12Q1/68
G01N33/68 C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,94 24285 (CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED) 27 October 1994 ---	
A	GENOMICS, vol. 24, 15 November 1994, pages 276-279, XP000570340 K. SUDO ET AL: "2058 expressed sequence tags (ESTs) from a human fetal lung cDNA library" -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
14 May 1996	18.06.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Le Cornec, N

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In ation on patent family members

Internatc Application No
PCT/US 96/00920

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9424285	27-10-94	EP-A- 0696321	14-02-96